intracellular environment (most particularly nuclease resistant), phosphorothioates are preferred for this purpose; and (c) the peptides (or other diverse chemical entities) are attached to the bead support via a linker that cleaves in the intracellular environment. Such linkers include linkers that can be cleaved upon the application of an external factor, such as light, that does not harm the cells and linkers labile to the intracellular environment, such as a phosphodiester bond or a disulfide bond, but in any case, the cleavable linker must be stable to the parallel synthesis process.

The library beads are introduced into the reporter cells preferably by a mechanical process such as, for example, biolistic projection. In some cases, a biochemically-mediated process leading to internalization can be employed, but this route usually results in incorporation into an undesirable cellular compartment (i.e., lysozomal localization). Once the beads are in the cells, the peptides or other compounds of interest are released. Given that 10 μm beads have a demonstrated capacity of 10^{10} peptide (or other) synthesis sites, then if the capacity scales with volume, a 1 μm bead of the same material would contain 10^7 molecules of the synthesized peptide. If all of the synthesized peptide is released in a single (spherical) cell of ~10 μm diameter (a volume of ~0.5 pL), then a concentration of free peptide of ~30 μM would result. This concentration would be controllable by the synthesis density on the beads, and a lower loading density could provide for a more stringent screening format (i.e., a screen for more active compounds). The recipient cells are engineered to generate a fluorescent signal upon activation or inactivation of the pathway of interest. The individual cells producing the desired effect are selected by FACS instrumentation, and the tags, which are still attached to the beads and contained within the cells, are amplified and sequenced to identify the active synthetic compounds.

In another embodiment, large beads are employed and used to screen a population of cells that express a receptor (i.e., the enzyme beta-galactosidase) capable of generating a fluorescent or other detectable signal, i.e., by cleavage of a substrate to produce a detectable compound. The beads are then mixed with a population of the cells, which are allowed to attach to the beads. If the receptor on the cell surface is stimulated by the compound on the bead, then the detectable compound is produced, providing a basis for sorting activated cells attached to the beads from unactivated cells. One could employ appropriate reagents (i.e., free labeled or unlabeled receptor) to maximize selection of high affinity ligands.

There are of course a variety of alternatives to flow cytometry for purposes of screening and selecting for library molecules of interest. In one embodiment, an encoded synthetic library is screened for antimicrobial activity to find compounds that retard the growth or kill bacteria or any other microorganism that can be plated in two dimensions, such as virus-infected cells, many eukaryotic cells including cancer cells, and some protozoa. Large libraries of related or unrelated chemical structures can be screened against cells in agar culture by controlled release of the peptide or compound from the bead on which it was synthesized.

The steps of the method follow: (1) plate the cells of interest on agar plates; (2) overlay the cells with another layer of agar in which the beads bearing the synthesized peptide/drug are suspended at a dilution that provides for even dispersion so that individual beads can be picked, e.g., with a capillary tube, from the solid agar; (3) initiate release of the peptide/drug from the beads; (4) culture the plate to allow diffusion of the peptide/drug from the bead immobilized in agar into the surrounding agar and into the agar below containing the indicator cells; (5) read the extent to which the diffused test compounds from individual beads have affected the growth/morphology/phenotype of the indicator cells; (6) choose zones where the indicator cells exhibit the desired response (e.g., death of a bacterial lawn) and using a capillary tube or similar, pick out the zone of agar that contains the original bead from which the test drug had diffused; (7) read the tag, e.g. by PCR amplification of the encoded material on the individual bead, to determine the structure of the peptide/drug that elicited the desired response; and (8) optionally chemically synthesize the appropriate drug/peptide and verify desired effect.

There are a variety of ways to release the test compound from beads. For instance, one could partially cleave the peptides/drugs from beads using TFA and allowing cleaved peptide to dry down onto the bead surface in such a form that subsequent resuspension in water (agar) will allow release of the peptide/drug and localization of the released compound to the zone of agar around a particular bead. One could link the drug/peptide to the bead using chemistry that is sensitive to a particular change in bead environment that can be initiated upon plating onto the agar and indicator cells or after plating and agar solidification, e.g., a photosensitive linkage, a thiol sensitive linkage, a periodate sensitive linkage, etc. These chemical agents could themselves be diffused in through another thin agar overlay, if necessary. Such release chemistry must be compatible with the integrity of the test substance, integrity of the encryption on the bead, and health of the underlying indicator cells. The particular release chemistry used will also of course depend on the type of chemistry used for synthesizing the library and the nature of the indicator cells. The method is especially preferred for screening libraries of beta-lactam antibiotics for identification of new antibiotics that might kill newly evolved strains of bacteria resistant to existing beta-lactams and for screening peptide libraries of analogues of known anti-bacterial peptides such as the magainins.

Other methods can also be used to screen bead-based molecular libraries. Affinity adsorption techniques can be employed in conjunction with the libraries of the invention. For example, the mixture of beads can be exposed to a surface on which a receptor has been immobilized (see PCT patent publication No. 91/07087, incorporated herein by reference). After washing the substrate to remove unbound beads, one can then elute beads bound to the surface using conditions that reduce the avidity of the oligomer/receptor interaction (low pH, acid treatment, or base treatment, for example). The process of affinity adsorption can be repeated with the eluted beads, if desirable. These methods, and related variants, such as the use of magnetic selection, described above, can be practiced in diverse ways; for instance the solid support can be a resin packed into a chromatographic column.

In another method of the invention, libraries of "tethered" compounds are used as a source of structural diversity in a form suitable for affinity purification of families of related molecules, such as families of receptors of pharmacologic interest. In general, this method relates to the use of a tagged and tethered molecular library to screen a second library of untagged molecules. The tagged, tethered library molecule serves as an affinity purification reagent to screen complex mixtures of soluble proteins, oligonucleotides, carbohydrates, antibodies, etc. Subsequent to affinity purification, molecules that bind to the combinatorial library members are identified by elution and appropriate separation and identification methods. The combinatorial library is then

divided into smaller fractions of combinatorially synthesized compounds to determine, through repeated cycles as necessary, reductively and precisely which compound(s) mediate the binding process.

In similar fashion, combinatorial chemical libraries can be used to identify and clone novel receptors. Many receptors are members of families of proteins that share sequence homology (usually reflecting divergent evolution from an ancestral parent) but exhibit differences in their specificity/affinity for structurally related sets of ligands/cognate receptors. Each member of a receptor family (R_n) may represent a separate target for specific pharmacologic action and hence for drug discovery and development by virtue of their different properties, i.e., locations in the body, specificities, affinities for ligands, etc. If one identifies a receptor (R_1) whose binding properties are of sufficient interest so that the identification of other receptors in the same family would be beneficial, then one can employ the following method to identify receptors related to R_1 in their binding site properties. One first identifies a ligand that binds to R_1 and then creates a tagged combinatorial compound library of molecules closely related structurally to the ligand.

Next, one prepares polysome preparations from cells believed likely to express additional members of the receptor family. Such polysomes comprise ribosomes attached to mRNA with pendant receptor in various stages of protein synthesis from nascent peptide to almost fully elaborated protein. The receptor protein nearing completion of synthesis will express the specific receptor property of binding to one or more members of the combinatorial library. Using the combinatorial library tethered to solid support, affinity purification of polysomes bearing receptors with affinity for any member of the combinatorial library is performed. Such affinity purification may involve column chromatographic methods, batchwise separation of immobilized components from the liquid phase, or aqueous two phase separation methods to achieve separation of the solid phase bearing attached receptor and relevant mRNA encoding the receptor from non-adherent polysomes.

Next, one performs cDNA synthesis from the mRNAs that encode the cognate receptor population using standard technology (reverse transcriptase, etc.) and clones the cDNA population into a vector suitable for rapid sequence analysis. Dependent on prior knowledge of the receptor sequences that are likely and the degree of sequence conservation that can be anticipated, one may attempt to use PCR or another amplification to amplify the cDNAs enriched by this method. By sequence analysis of a suitable number of cDNA clones, one can identify cDNAs (whether full length or not) that show sufficient sequence homology with the sequence of the already known receptor R_1 to represent putative additional members of the same receptor family (R_n). One prepares optionally full length cDNA clones of these novel cDNAs (or relevant portions thereof, such as the portion encoding the extracellular domain of relevance to ligand binding) by standard cloning methods and expresses these cDNAs by standard methods (i.e., in eukaryotic expression systems as soluble or membrane bound proteins as appropriate). Using standard formats for testing receptor ligand interaction, one tests for binding of populations of mixed compounds from the combinatorial library or individual compounds. In this way, one can identify precisely which compound(s) from the library bind to the newly identified receptor.

Individual beads can be physically separated, for example, by limited dilution or by methods similar to those in which cells are incubated with a receptor coupled to small superparamagnetic beads and then cells expressing a ligand for the receptor are extracted using a high power magnet (see Miltenyi et al., 1990, *Cytometry* 11: 231–238 incorporated herein by reference). As noted above, magnetically selected cells can be further analyzed and sorted using FACS. Radionucleotides may also serve to label a receptor, allowing one to identify and isolate beads by selecting beads that are radioactively labeled.

B. Screening Soluble Molecules

One can also employ tagged molecular libraries to useful effect in novel assays of the invention in which a ligand is solubilized in either tagged or untagged form prior to binding to a receptor of interest. For screening very large libraries of soluble (bead-free) tagged molecules, one preferably employs affinity chromatography under conditions of weak affinity. For example, a 30 mg library of 10^{18} molecules can be screened with a simple 10 mL affinity chromatography column containing a few hundred μg of a receptor of interest. Oligonucleotide are preferred tags for such libraries, being readily PCR amplified and cloned into the commercially available TA cloning vector (Invitrogen, Inc.), a convenient form for storing tag information prior to analysis by DNA sequencing. In addition, oligonucleotide tags can be concatenated, as described above, allowing one to collect pools of soluble tagged molecules, clone the concatenated tags from the selected pools, and then sequence the tags to identify the desired compounds.

Soluble tagged molecules can also be screened using an immobilized receptor. After contacting the tagged molecules with the immobilized receptor and washing away non-specifically bound molecules, bound, tagged molecules are released from the receptor by any of a wide variety of methods. The tags are optionally amplified and then examined and decoded to identify the structure of the molecules that bind specifically to the receptor. A tagged oligomer in solution can be assayed using a receptor immobilized by attachment to a bead, for example, by a competition assay with a fluorescently labeled ligand. One may recover the beads bearing immobilized receptors and sort the beads using FACS to identify positives (diminished fluorescence caused by the library molecule competing with the labeled ligand). The associated identifier tag is then be amplified and decoded.

The soluble molecules of the library can be synthesized on beads and then cleaved prior to assay. In one embodiment, the microscopic beads of a molecular library are placed in very small individual compartments or wells that have been "nanofabricated" in a silicon or other suitable surface. Beads are loaded in the wells by dispersing them in a volume of loading buffer sufficient to produce an average of one bead per well. In one embodiment, the solution of beads is placed in a reservoir above the wells, and the beads are allowed to settle into the wells. Cleavage of the oligomers from the beads may be accomplished using chemical or thermal systems, but a photocleavable system is preferred. The molecules of interest can be cleaved from the beads to produce either untagged molecules in solution (the tag remaining attached to the bead) or tagged molecules in solution. In either event, the molecules of interest are cleaved from the beads but remain contained within the compartment along with the bead and the identifier tag(s).

In one embodiment, a surface or a portion of the surface of the well is coated with a receptor. Binding buffer and a fluorescently labeled known ligand for the receptor is added to the well to provide a solution phase competition assay for ligands specific for the receptor. The binding of the fluorescently labeled ligand to the receptor can in one embodiment

be estimated by confocal imaging of the monolayer of immobilized receptor. Wells with decreased fluorescence on the receptor surface indicate that the released ligand competes with the labelled ligand. The beads or the tags in wells showing competition are examined to reveal the identity of the competitive ligand.

Recovery of identifier-tagged beads from positive wells may optionally be effectuated by a micromanipulator to pluck individual beads out of wells. Another mode involves the use of beads that have incorporated a fluorescent molecule, either during bead manufacture or through labeling. A laser of the appropriate wavelength is used to bleach the resident beads in only the positive wells. All the beads are then removed en masse and sorted by FACS to identify the bleached positives. The associated tags may then be amplified and decoded to identify the molecules that bind specifically to the receptor.

In another embodiment of the invention, one employs relatively large tagged beads, from which the molecules of interest are cleaved in a series of reactions. In this method, the beads are 50 to 500 μm in diameter, with capacities equivalent to 100 to 500 pmol of peptide per bead; preferably, one uses 100 μm beads with a capacity of about 200 pmol, if constructing a peptide library. The typical size of such a library is from about 10^6 to 10^8, preferably 10^7 different molecules. The library is divided into about 100 pools, each containing about 100,000 beads. A certain percentage, about 25%, of the molecule of interest is cleaved from the pool, producing, in the case of a peptide library, for example, each peptide at 50 nM in a volume of 1 mL.

The cleaved pool is then tested in a competition or functional assay. One identifies the pools with the highest activity, and then retrieves the remainder of the original pool and aliquots the remainder into 100 pools of 1000 beads per pool. The process is repeated until one has a single bead, from which one reads the tag and identifies the compound of interest. This method avoids the resynthesis and frame limitations of the Houghten method and is advantageous in that the pools are random, rather than related, compounds. The chances of a mixture being active because of the cumulative potency of many low affinity related molecules is reduced.

C. Screening Natural Product Libraries

With the automated high flux assays that are now available, the present limitations in natural product screening are first, the ability to obtain and handle (dispense, dissolve, label, etc.) the samples; and second, the substantial effort required to characterize the active components of positive samples. The present invention provides methods for generating and screening natural product libraries that can provide a huge number of samples in readily screened form and to identify active components in the samples. The basis of the method is the combination of biochemical and chemical diversity with metabolic diversity from "natural products", i.e., from nature. The simplest example involves feeding collections of peptides to cultures of microorganisms. Each microbial strain might create many modified peptides (a metabolite library). Because each culture would (potentially) contain a very complex mixture of metabolites, an efficient method of screening is required.

Several approaches are available and might be orthogonally classified as factored or tagged, and soluble or tethered. For the sake of illustration, consider as the "feedstock" a library of soluble peptides. An aliquot of the library is incubated with each of the many strains typical of a microorganism fermentation screening program, and the media screened in typical fashion. Positive cultures are then incubated with subsets of the libraries and rescreened. This process of factoring continues until the input_peptides generating the most active metabolites are identified. The characterization of the active metabolites then proceeds aided by the knowledge of the likely precursor molecules. Thus, the first screening identifies the active organism(s), subsequent steps identify the active precursors, and finally, the active metabolites are identified by standard analytical means.

In all its formats, however, factoring is a tedious process. Libraries produced by split synthesis and cleaved free of the resin produce soluble compounds amenable to cellular uptake and metabolism by intact organisms. However, the concentrations of the individual compounds is quite low (inversely related to the diversity of the collection), leading to inefficient enzymatic turnover and very low concentrations of the resulting metabolites. The concentrations of the compounds may be increased by producing subsets of the libraries and fermenting each subset separately with each microbial isolate. Sub-libraries are constructed by fixing one or more of the positions and randomizing the remaining positions. For example, there are 500 pentapeptide sublibraries containing all permutations of 2 fixed positions utilizing 50 building blocks. Each of these sublibraries contains 125,000 compounds. The use of tagged libraries offers a major advantage in ease and sensitivity, but requires modifications in the method of exposing the compound collections to the metabolic activities. The combinatorial feedstock need not be only peptides but could consist of any type of combinatorial chemical collections.

Oligomer and other molecular libraries can be constructed in a combinatorial process and each step encoded with identifying tags. This may be done via a direct linkage and parallel synthesis of the oligomer to the tag. If oligonucleotides are used as the tags, then the complexes will be relatively large but small enough to insert actively into the cells via liposome fusion, electroporation, solvent permeabilization, etc. Once inside, the complexes would be subject to the metabolic machinery of the cells. One would avoid the vulnerability of the oligonucleotide tags to degradation by the use of modified nucleotides and nucleotide linkages. Upon recovery of the active metabolites from the culture of from lysed cells, the samples are screened and the tags decoded to reveal the precursor compound. Scaled-up fermentation of the active organism with the active precursors should produce sufficient quantities of the active metabolites to characterize. Libraries of compounds made by an encoded combinatorial process on beads can be exposed to lysates of bacteria, fungi, plant cells, etc. With this format, the need to insert the tagged complexes into intact cells is avoided, and only a relatively few of the many molecules on the bead need be processed to be detected (e.g., in a fluorescence-activated binding asay).

Another useful method of the invention involves the utilization of the products of one microbial culture as feed for another culture. To illustrate, consider a collection of 100 different microbial isolates from large scale cultures (~1 liter). The supernatant of each culture is recovered by filtration and divided into one hundred 10 mL aliquots. Each aliquot is inoculated with one of the 100 strains and incubated. 10,000 samples (metabolites of metabolites) are thereby generated from the 100 microbial isolates. This method of combinatorial metabolism can be extended to sequential metabolism by greatly different species: subjecting the product of microbial fermentation to incubation with exotic plant lysates or incubating extracted fractions of plant tissues with fungal cultures, for example. These methods can

graphic separation of a natural product extract is now able to act at any point in the intracellular signal transduction pathway. The fusion step also adds the specific tags that provide the signature of the particular test compound sample to the individual acceptor cell. If those tags were in the lipid membrane of the liposome, then the tags are distributed in the outer cell membrane of the acceptor cell. Antigens at this location are accessible to panels of specific monoclonal antibodies. Rare earth metal ions that were in the aqueous phase of the particular liposome are in the acceptor cell cytoplasm. The fusion step also adds the shared liposome tag that identifies cells that acted as acceptors from those, the excess, that did not undergo a liposome fusion event. The tag can be a fluorophore that moves from the liposome membrane to the acceptor cell membrane.

The mixture of cells, cells fused to individual liposomes, and any unfused liposomes is next incubated with the exogenous ligand (e.g., in the case of testing for an antagonist) or incubated without any addition (e.g., in the case of testing for an agonist). The time of this incubation is determined using control compounds at defined concentrations and incubation times.

Preferably, one uses FACS to select compounds (cells) of interest. For instance, one can first use forward or side light scatter to sort cells (whether acceptors or not) from any unfused liposomes. Large cells can be readily separated from small liposomes. Next, one can sort cells that were liposome acceptors from those, the excess, that were not liposome acceptors. Cells that were acceptors bear the shared liposome-derived fluorescent label, whereas the non-acceptor cells are non-fluorescent with this label. This step is of course optional but, if performed as a presort, allows separation of the (typically) majority of cells that are irrelevant to subsequent analysis from the minority that were acceptors. For identification of an antagonist, one can sort on the basis of light emission from the reporter protein (e.g., beta-galactosidase or luciferase), separating the majority of fluorescence-positive cells (rendered such by addition of the exogenous ligand earlier), from the minority of fluorescence-negative cells or low fluorescence cells. The latter two cell categories result from presumed antagonist effects of compounds that were encapsulated in the particular liposomes that fused with these individual cells. For identification of an agonist, one can sort on the basis of light emission from the reporter protein, separating the majority of fluorescence-negative cells from the minority of fluorescence-positive cells. The latter cells have resulted from a presumed agonist effect of liposome-derived compounds.

In some experiments, one can sort all cells of interest according to the criteria above as a population and collect occasional cells as cloned individuals using standard FACS methods. These individual cells can be analysed as single cells for the particular tags that they bear, allowing precise identification of the particular liposome that mediated the desired effect. In other applications, one can analyse the tag distribution in the entire sorted event-positive population and dependent on the design of the experiment and particular tags that had been incorporated in samples from different times/locations/inventories, be able in a first pass to determine the diversity of tag types in the total event-positive population.

Collected single cells or populations of cells can be analysed by methods appropriate to the particular tag combinations used. Fluorescence tags can be analysed by FACS and/or traditional spectrophotometry. Antigen tags can be analysed by addition of appropriately labelled monoclonal antibodies and ELISA, FACS, radioisotopic, or luminescence assisted assays. Metal ion tags can be analysed last by atomic absorption spectrometry. After tag decoding, the tests can be repeated either with mixtures of only those liposomes that yielded a positive event on first pass or with pure liposomes of each member of interest added to separate cell samples.

These and other methods of the invention can be automated to facilitate practice of the invention, as discussed in the following section.

VII. Instrumentation

The coupling steps for some of the monomer sets (amino acids, for example) can in some embodiments require a relatively lengthy incubation time, and for this and other reasons a system for performing many monomer additions in parallel is desirable. The present invention relates to automated instrumentation for use in generating and screening encoded synthetic molecular libraries. One preferred instrument, able to perform 50 to 100 or more parallel reactions simultaneously, is described in U.S. patent application Ser. No. 08/149,675, filed herewith at even date in the name of inventors J. Sugarman et al. (Attorney Docket No. 1007.3), incorporated herein by reference. Such an instrument is capable of distributing the reaction mixture or slurry of synthesis solid supports, under programmable control, to the various channels for pooling, mixing, and redistribution.

In general, however, the instrumentation for generating synthetic libraries of tagged molecules requires plumbing typical of peptide synthesizers, together with a large number of reservoirs for the diversity of monomers and the number of tags employed and the number of simultaneous coupling reactions desired. The tag dispensing capability translates simple instructions into the proper mixture of tags and dispenses that mixture. Monomer building blocks are dispensed, as desired, as specified mixtures. Reaction agitation, temperature, and time controls are provided. An appropriately designed instrument also serves as a multichannel peptide synthesizer capable of producing 1 to 50 mgs (crude) of up to 100 specific peptides for assay purposes. See also PCT patent publication 91/17823, incorporated herein by reference.

Typical instrumentation comprises (1) means for storing, mixing, and delivering synthesis reagents, such as peptide and oligonucleotide synthesis reagents; (2) a sealed chamber into which the various reagents are delivered and inside of which the various reactions can proceed under an inert atmosphere; (3) a matrix of sealed reaction vessels; (4) means for directing the flow of reagents to the appropriate reaction vessels: (5) means for combining and partitioning small (0.1–100 μm) beads; and (6) means for washing the beads in each reaction vessel at the conclusion of each chemical reaction. The matrix of reaction vessels can have any one of several designs. For example, the vessels can be arranged in a circle so that the vessels can be made to rotate about a central axis (i.e., a centrifuge). Alternatively the vessels can be arranged in a 12×8 matrix (96-well microtiter plate format). Any arrangement amenable to accessibility by robotic delivery, aspiration, and transfer functions is useful for some applications.

The system used for combining and redistributing particles can have one of several designs. For instance, the beads can be suspended in a solvent of appropriate surface tension and density such that a robotic pipetting instrument can be used to transfer the beads to a combining vessel. After mixing, the beads can be redistributed to the reaction vessels by the same robotic pipettor. Alternatively, the beads can be combined by using a special valved reaction chamber. The

valve is opened to allow solvent flow to transfer the beads to a combining vessel. After mixing, the beads are repartitioned by reversing the flow to each reaction vessel.

In another embodiment, the beads are combined using closely spaced reaction vessels with open top ends. Flooding the vessels allows the beads to mix. If the beads are magnetic, then the beads are re-partitioned by pulling the beads back down to the bottom of the vessels by application of a magnetic field. Non-magnetic beads are re-partitioned by vacuum suction through the bottom of the reaction vessels. In yet another embodiment, the beads are partitioned by distributing them on a flat surface and then restricting them to certain sectors by covering them with a "cookie-cutter" shaped device, described more fully below.

The system for washing the beads can also have one of several designs. The beads can be washed by a combination of liquid delivery and aspiration tubing. Each reaction vessel has its own set of tubing, or a single set can be used for all reaction vessels. In the latter case, the liquid delivery and aspiration lines can be mounted on a robotic arm to address each vessel individually. The beads in each vessel can be made to form a single pellet by either centrifugation or the use of magnetic beads and application of a magnetic field. One can also employ a reaction vessel with a bottom wall composed of a chemically inert membrane so that reagents can be removed from the vessels by application of a vacuum. Reagents can also be removed from each vessel by using vessels that can accommodate continuous flow through of reagents and washing solutions, i.e., a vessel with luer fittings and membranes on each end.

Any automated combinatorial instrument that relies on an individual reaction chambers, each connected to reagent delivery systems and to a "mother pot" to which the beads are pumped for pooling and from which the beads are reallocated among the reaction chambers for successive rounds of monomer addition faces a very important practical limitation. There is a wealth of monomer or other building block units, and the difficulty of partitioning beads and reagents among the potentially large number of reactions may limit such instruments to fewer than 100 separate parallel reactions.

The present invention provides an instrument that avoids the need to pump beads between chambers to mix and reallocate, simplifies reagent delivery, and allows the simple and accurate partitioning of very small numbers of tiny beads. The basic design consists of a plate with an array of reaction "sites" located on the surface; the surface may be planar or may consist of an array of shallow wells that form reaction sites. In one embodiment, there are 256 sites in a 16×16 array. Each reaction site is a spot, or well, on the surface to which a group of synthesis beads is attracted. The attractive force may be magnetism, vacuum filtration, gravity with passive mechanical sorting, or various other simple means. The beads are initially applied as a dilute slurry in a shallow reservoir evenly covering the array of reaction sites. Upon application of the attractive force, beads are concentrated at each site.

After positioning all the beads on the reaction sites, the sites are then separated by mechanical partitions to create (temporarily) the individual reaction chambers as shown in FIG. 1. A variation provides partitions permanently affixed to the surface to form shallow wells. The reaction components are delivered to each chamber, the beads released into suspension, and the reaction initiated. When desired, the beads can be reattached to the surface and the reagents removed. After all steps for a coupling cycle are completed, the chamber partitions are removed, and the beads are released into the common reservoir above the array of sites.

Mixing of the beads is caused by induced convection of the reservoir fluid, and the beads are then reattracted to the surface sites for the next round of coupling. Subsequent steps, including the wash steps, are accomplished in a similar fashion. Addition and removal of reagents is done with a combination of plumbing and automated pipetting. Addition of reaction specific reagents (monomers, for example) may be done with robotic multipipettors. Addition of common reagents and the removal of all reagents can be done with a fixed plumbing system not requiring valving at each reaction chamber. Some common steps such as washes can be done on the beads en masse, before installing or after removing the chamber partitions.

The use of large numbers of monomers or other building blocks places an additional burden on the encoding process. In one encoding scheme for oligonucleotide tags, a basis set of 1000 monomers might require a 5 base sequence to tag each reaction step; a set of more than 1024 monomers could require 6 bases to encode. To reduce the plumbing complexity of the synthesis instrument (i.e., to reduce the number of specific reaction additions), a special encoding strategy is provided by the present invention. To illustrate the method, consider an array of 16×16 reaction sites, an arrangement that allows 256 different reactions to be carried out simultaneously. To encode each reaction individually with multiple base coupling is a difficult undertaking.

The array consists of 16 rows and 16 columns, each site in the array having a unique geographical address. Each row of sites can be tagged as a group, and all 16 rows can be uniquely encoded with 2 base codons ("subcodons"). A striped template or channel block can serve to form the 16 reaction chambers for these 2-base additions (note that the bases are coupled as monomeric phosphoramidites, not as dimers). See PCT patent publication No. WO 93/09668, incorporated herein by reference. This form of addressing of the reaction sites is analogous to others; for example, an optical method can be used to label the beads, as in the striped masking process described in U.S. Pat. No. 5,143,854, incorporated herein by reference. If a template or channel block is employed, then the template is lowered onto the synthesis surface just as is the grid template that isolates the individual reactions during synthesis of the library molecule.

The beads are not released during the tagging reaction, however, as their spatial segregation must be maintained through the next step. When the rows have been tagged with subcodons, the template is lifted, rotated 90°, and lowered to form stripes covering the columns. The 16 columns are then labelled with 2-base subcodons, resulting in the unique tagging of each of the 256 reaction addresses with a 4-base "supercodon". By identifying the reaction addresses, the supercodons also specify the monomer that was added in each reaction.

The Example that follows illustrates an improved method of the invention for making peptide libraries tagged with oligonucleotide tags.

EXAMPLE 1

LIBRARY PREPARATION AND SCREENING

This Example illustrates how the products of a combinatorial peptide synthesis on resin beads can be explicitly specified by attaching an oligonucleotide identifier tag to the beads coincident with each amino acid coupling step in the synthesis. Each tag conveys which amino acid monomer was coupled in a particular step of the synthesis, and the overall sequence of a peptide on any bead can be deduced by

reading the tag(s) on that bead. The collection of beads can be screened for binding to a fluorescently-labelled anti-peptide antibody using a fluorescence activated cell sorting (FACS) instrument. Those beads to which an antibody binds tightly can be isolated by FACS, and the oligonucleotide identifiers that are attached to individual sorted beads can be amplified by the PCR. The sequences of the amplified DNAs are determined to reveal the identity of the peptide sequences which bind to the antibody with high affinity. By combining high capacity, oligonucleotide code-based information storage, amplification methodology, and fluorescence-based sorting, the present method provides a means for specifying the identity of each member of a vast library of molecules synthesized from both natural and unnatural chemical building blocks and for quickly and efficiently isolating individual beads that bear high affinity ligands for biological receptors.

In this Example, single stranded oligonucleotides are used to encode a combinatorial peptide synthesis using both L- and D-amino acid building blocks and 10 μm diameter polystyrene beads. The oligonucleotide tags have a high information content, are amenable to very high sensitivity detection and decoding, and, with the present method, are stable to reagents used in peptide synthesis. Peptides and nucleotides are assembled in parallel, alternating syntheses so that each bead bears many copies of both a single peptide sequence and a unique oligonucleotide identifier tag. The oligonucleotides share common 5'- and 3'-PCR priming sites, and thus the beads can serve as templates for the PCR. The encoded synthetic library contains about 8.2×10^5 hepta-peptides and is screened for binding to an anti-dynorphin B monoclonal antibody D32.39 (see Barrett & Goldstein, 1985, *Neuropeptides* 6: 113–120, incorporated herein by reference), using a fluorescence activated cell sorting (FACS) instrument to select individual beads that strongly bind the antibody. After PCR amplification of the oligonucleotide tags on sorted beads, the DNA is sequenced to determine the identity of the peptide ligands.

A. Reagents and General Methods

The monodisperse 10 μm diameter bead material used in this work was a custom-synthesized macroporous styrene-divinylbenzene copolymer functionalized with a 1,12-diaminododecane linker purchased from Pharmacia. The beads are Pharmacia Monobeads™ that have not been derivatized with Pharmacia's Gene Assembler Support linker. See Ugelstad and Mork, 1980, *Adv. Colloid. Interface Sci.* 13: 101–140, incorporated herein by reference.

All protected amino acids were obtained from Bachem Bioscience Inc. PCR and sequencing primers were synthesized with an Applied Biosystems model 394 oligonucleotide synthesizer. Authentic samples of certain peptides were synthesized with an Applied Biosystems model 431A peptide synthesizer using Fmoc-protected amino acids, HBTU/HOBt in situ activation chemistry, and deprotection with 40:1:1 TFA/water/ethanedithiol. These peptides were purified by HPLC (>95% purity) on a Rainin C_{18} reverse phase column using water/acetonitrile/0.1% TFA as eluant, and structures were verified by mass spectrometry.

B. Parallel Synthesis of a 69-base Oligonucleotide and the Opioid Peptide Dynorphin B

The C-terminal seven amino acid fragment of the opioid peptide dynorphin B H-Arg-Gln-Phe-Lys-Val-Val-Thr-NH_2 (RQFKVVT) (SEQ ID NO:2) was synthesized in parallel with a 69-mer oligodeoxynucleotide (ST08) on 10 μm diameter beads. The sequence of ST08 was 5'-ATC CAA TCT CTC CAC (ATC TCT ATA CTA TCA) TCA CC [TA TC CT AT TT TT AC] CTC ACT CAC TTC CAT TCC AC-3' (SEQ ID NO:20). Underlined portions of this sequence correspond to PCR-priming sites while the region in parentheses is homologous to the primer used for sequencing this template. The 14-base sequence enclosed in brackets represents the coding region of the template.

The beads were first treated with a mixture of succinimidyl 4-O-DMT-oxybutyrate (Molecular Probes) and the 1-oxybenzotriazole ester of either N-Fmoc-2,4-dimethoxy-4'-(carboxymethyloxy)-benzhydrylamine (i.e. the acid-cleavable Knorr carboxamide linker) or N-Fmoc-Thr(tBu)-OH (for non-cleavable experiments). The ratio of Fmoc-protected amino groups to DMT-protected hydroxyl residues on the beads was determined spectrophotometrically to be approximately 20:1. The beads were subjected to 20 cycles of oligonucleotide synthesis on an automated synthesizer using 3'-O-methyl-N,N-diisopropyl phosphoramidites of the following nucleosides: N^6-Bz-5'-O-DMT-(7-deaza)-2'-deoxyadenosine (Berry and Associates, Ann Arbor, Mich.), N^4-Bz-5'-O-DMT-2'-deoxycytidine, and 5'-O-DMT-thymidine (Glen Research).

The beads were then removed from the instrument and treated for 5 min. with 10% piperidine in DMF to remove the Fmoc protecting group. After coupling the first amino acid residue (N-Fmoc-Thr('Bu)-OH), the beads were treated with a DMF solution of acetic anhydride and 1-methylimidazole to cap any unreacted amines. All peptide coupling reactions were run for 20 min. and contained 0.11M Fmoc-amino acid, 0.1M HBTU, 0.1M HOBt, and 0.3M DIEA in DMF. The beads were then subjected to two cycles of nucleotide addition on the synthesizer (detritylation with TCA; tetrazole-catalyzed phosphitylation; capping with acetic anhydride; oxidation with iodine in acetonitrile/water). Sequential steps of amino acid coupling and dinucleotide addition were repeated until synthesis of the peptide sequence RQFKVVT (SEQ ID NO:2) and construction of the oligonucleotide coding region had been completed. After performing an additional 35 cycles of oligonucleotide synthesis, the beads were treated sequentially with piperidine/DMF (1:9 for 8 min), thiophenol/triethylamine/dioxane (1:2:2 for 4 hr), ethylenediamine/ethanol (1:1 for 5 hr at 55° C.), and TFA/water (20:1 for 1 hr) to deprotect fully both the peptide and oligonucleotide chains. In experiments using the acid-cleavable linker, the supernatant from the TFA deprotection reaction was concentrated in vacuo, and the isolated crude peptide was then analyzed by HPLC.

C. Construction of an Encoded Library

The parallel synthesis chemistry outlined above was used in the construction of the library. The sites of peptide synthesis were differentiated from DNA synthesis sites in this experiment by coupling to all the beads a mixture of N-Fmoc-Thr('Bu)-OBt and succinimidyl 4-O-DMT-oxybutyrate as described above. Sequences of oligonucleotide tags in the library deviated from ST08 only within the coding region. The 3'-conserved region of the oligonucleotide ST08 was first synthesized on a total bead mass of 35 mg ($\sim1.75\times10^8$ beads). The Fmoc protecting group was removed and the bead mass was divided into seven equal parts. To each aliquot was coupled one of seven different alpha-N-Fmoc-protected amino acids (side chain protecting groups are shown in parenthesis): Arg(N^G-Pmc), Gln(Trt), Phe, Lys('Boc), Val, D-Val and Thr('Bu). Each part was then subjected to two rounds of automated oligonucleotide synthesis. The respective sequences of the appended dinucleotides that specified uniquely each different amino acid residue were TA, TC, CT, AT, TT, CA and AC. The beads were then pooled, mixed thoroughly, and the entire bead mass subjected to Fmoc deprotection.

This cycle of bead partitioning, peptide coupling, oligonucleotide dimer synthesis, bead recombination and Fmoc removal was repeated for a total of seven times. The final Fmoc protecting group was not removed. Rather, the pooled bead mass was subjected to 35 cycles of oligonucleotide synthesis. The library was then fully deprotected as described above.

D. Library Staining and FACS Analysis

A portion of a library (typically 0.5–2 mg of beads) was suspended in blocking buffer (PBS, 1% BSA, 0.05% Tween-20) and incubated at room temperature for 1 hr. The beads were pelleted by centrifugation and resuspended in a solution of mAb D32.39 (10 mg/mL in blocking buffer). The suspension was incubated on ice for 30 min., pelleted by centrifugation, and washed with blocking buffer. The beads were then suspended in a solution of phycoerythrin-conjugated goat anti-mouse antibody (Molecular Probes) for 20 min. on ice. The beads were washed in blocking buffer and diluted in PBS for delivery into the fluorescence activated cell sorting (FACS) instrument (Becton Dickinson FACStar Plus). Beads which had bound the mAb D32.39 were identified by their acquired fluorescence. Individual beads from both the most brightly stained 0.17% of the library and from the region having the lowest fluorescence (ca. 98%) were sorted into PCR microfuge vials. Specific binding of D32.39 to the beads was blocked by preincubation of the mAb with the soluble peptide Ac-RQFKVVT-OH (SEQ ID NO:2) at a final concentration of 10 μM.

E. PCR of Bead-Bound Template

PCR amplifications were performed in the manufacturer supplied buffer system (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 2 mM $MgCl_2$) with 0.2 mM dATP, dCTP, and dGTP, 0.8 mM dUTP, 2 mM each primer, 3 units Taq polymerase (Promega), and 1 unit of uracil DNA glycosylase (Gibco BRL) (total volume 70 μL). The primer sequences, 5'-ATC CAA TCT CTC CAC-3' (SP13) (SEQ ID NO:2) and 5'-(biotin)-GTG GAA TGG AAG TGA-3' (SP14) (SEQ ID NO:22) were respectively homologous and complementary to the template ST08. PCR reactions consisted of 45 cycles of denaturation at 95° C. for 30 sec., primer annealing at 50° C. for 1 min., and extension at 72° C. for 1 min. Reactions were analyzed by electrophoresis in 20% acrylamide or 2% low melting point agarose gels.

F. Sequencing of PCR Product

Biotinylated PCR product from individual reactions was isolated with streptavidin-coated magnetic beads (Dynal, Inc.). After alkaline elution of the non-biotinylated strand and washing, each bead sample was treated with sequencing cocktail. Dideoxy sequencing was performed using the primer 5'-ATC TCT ATA CTA TCA-3' (SP15) (SEQ ID NO:23) and Bst polymerase (Bio-Rad) according to the manufacturer's instructions, with the exception that a 1:100 ratio of deoxy- to dideoxynucleotide triphosphates (Pharmacia) was employed.

G. Determination of Peptide Binding Affinities

The binding affinities of various peptides for the monoclonal antibody D32.39 were measured in a competition binding experiment. A tracer peptide (LRRASLGGGRRQFKVVT (SEQ ID NO:24); 50 pM) containing the known epitope for D32.39 fused to a consensus substrate sequence for cAMP-dependent protein kinase was radiolabelled to high specific activity with [g-^{33}P]ATP (see Liet al., 1989, *Proc. Natl. Acad. Sci. USA* 86: 558–562, incorporated herein by reference) and mixed with various concentrations of the peptide of interest (10 μM–1 pM). The peptide mixtures were added to polystyrene wells coated with D32.39 (0.1 μg/mL). Samples were incubated 2 hr. at 4° C., the wells washed with PBS, and the radioactivity associated with each well was counted and used to generate a competitive binding curve. Under the conditions of the assay the IC_{50} should be close to the dissociation constant (Kd) for the peptide.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 24

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Arg Gln Phe Lys Val Val Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Thr Phe Arg Gln Phe Lys Val Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Thr Thr Arg Arg Phe Arg Val Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Thr Val Arg Gln Phe Lys Thr Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Gln Val Arg Gln Phe Lys Thr Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Arg Gln Phe Arg Thr Val Gln Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Gln Phe Lys Val Thr Lys Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Gln Gln Phe Lys Val Val Gln Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Lys Gln Phe Lys Val Thr Gln Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Thr Gln Phe Lys Val Thr Lys Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Thr Phe Arg Val Phe Arg Val Thr
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Phe Arg Arg Gln Phe Arg Val Thr
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Gln Phe Lys Gln Val Gln Thr
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Thr Val Thr Val Lys Lys Thr
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Gln Val Gln Arg Gln Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys Thr Gln Val Val Gln Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Val Thr Gln Val Arg Val Thr
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Phe Val Val Thr Val Arg Val Thr
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (oligonucleotide)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATCCAATCTC TCCACATCTC TATACTATCA TCACCTATCC TATTTTTACC TCACTCACTT 60

CCATTCCAC 69

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (oligonucleotide)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATCCAATCTC TCCAC 15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (oligonucleotide)

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGGAATGGA AGTGA 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (primer)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATCTCTATAC TATCA 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Arg Arg Ala Ser Leu Gly Gly Gly Arg Arg Gln Phe Lys Val Val
1 5 10 15

Thr

We claim:

1. A method of screening a tagged library of diverse compounds, wherein said library comprises a plurality of different members, each member comprising:

a solid support;

multiple copies of a compound bound to each of said supports, wherein the compound bound to one of said supports is different from the compound bound to selected other solid supports, and wherein said compound comprises a peptide; and

one or more identifier tags bound to each of said solid supports, wherein said tag identifies the compound bound to said solid support or identifies a reaction said solid support has experienced, and wherein said tag is an oligonucleotide or a fluorescent tag, said method comprising the steps of:

a) cleaving at least a portion of said compounds from said solid supports to yield a collection of untagged soluble compounds wherein said tags remain bound to said solid supports;

b) incubating said collection of untagged soluble compound s with a receptor under conditions conducive to binding of a ligand to said receptor; and

c. c) determining whether any compounds of said collection have bound to the receptor.

2. The method of claim 1 wherein said solid support is a bead 50 to 500 microns in diameter.

3. The method of claim 1, wherein said identifier tag is covalently attached to a first linker and said first linker is attached to said solid support and not to said compound.

4. The method of claim 1, whrein all of said compounds on said solid supports are cleaved prior to said incubation step.

5. The method of claim 1, further comprising the step of deducing the structure of the compounds that have bound to the receptor by examining the tags associated with the receptor-bound compounds.

6. The method of claim 1, wherein said receptor is a mixture of receptors and wherein each receptor bears a receptor-identifier tag that identifies the receptor.

7. The method of claim 1, wherein said receptor is immobilized on a second solid support.

8. The method of claim 7, wherein said second solid support is a surface or portion of the surface of a small individual compartment or well.

9. The method of claim 1, wherein said compound is attached to said solid support by a cleavable linker.

10. The method of claim 9, wherein said cleavable linker is a mixture of cleavable linkers.

11. The method of claim 10, wherein only a portion of said compounds on said solid supports are cleaved prior to said incubation step.

12. In a method of synthesizing a synthetic peptide library comprising a plurality of different members, each member comprising a peptide composed of a sequence of amino acid monomers linked to a bead to which bead is also linked one or more oligonucleotide identifier tags identifying the sequence of monomers in said peptide, wherein said amino acid monomers are protected with Fmoc and piperidine is used to remove the Fmoc protecting group, the improvement comprising effecting Fmoc removal by treatment with 5 to

15% piperidine for 5 to 60 minutes or 15 to 30% piperidine for 1 to 30 minutes.

13. The improvement of claim 12, wherein said bead is about 10 μm in diameter and composed of a macroporous styrene-divinylbenzene copolymer derivatized with a dodecylamine linker.

14. The improvement of claim 12, wherein said amino acid monomers have side ^{t}Bu side chain protecting groups, trifluoroacetic acid is used to remove said ^{t}Bu side chain protecting groups, and said oligonucleotide tags comprise 7-deaza-2'-deoxyadenosine.

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